DEDIFFERENTIATION IN ECHINUS LARVÆ, AND ITS RELATION TO METAMORPHOSIS.

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CONTENTS.

1. INTRODUCTION.

The following observations and experiments were made at the Marine Biological Association's Laboratory at Plymouth in July and August, 1920.

The accidental discovery that dedifferentiation, of a type similar to that already studied in *Clavellina,* occurred in Echinoderm larvæ led me to make further observations on this phenomenon. Owing to lack of material, they are very fragmentary. I hope to resume them at the earliest opportunity. Meanwhile their theoretical bearing on the question of metamorphosis warrants their publication in their present form.

I have to thank the Director and Staff of the Plymouth Laboratory for their assistance. The work was carried on with the aid of a grant from the Royal Society.

2. DIFFERENTIAL MODIFICATION OF GROWTH.

Experiments were started on fertilized eggs of *Echinus miliaris* in order to test certain conclusions of Child's ('16 and '17) as to

the effect of dilute poisons on differential modification of growth, and to compare the effects of cyanides and mercury salts.

A. When fertilized *Echinus* eggs were placed in KCN *n*/50,000[1] growth ceased at the 2- or 4-cell stage. A small percentage of eggs were cytolysed and many had multiple asters and irregular segmentation. In KCN *n*/100,000, after 24 hours more eggs were cytolysed, a certain proportion had got no further than the 2- or 4-cell stage, and a small percentage had become blastulæ, most of them of the abnormal solid type (stereoblastulæ). The controls had in the same period reached the gastrula stage.

After 72 hours, 24 hours after the controls had reached the early pluteus stage, these blastulæ had become late gastrulæ. These were transferred to sea-water, and developed as far as the pre-pluteus stage, but never became normal plutei, thus bearing out Child's conclusions that considerable poisoning in the early stages in some way disturbs the relations of parts so that even when the developing organism is replaced in normal conditions, it can only develop up to a certain stage, and no further.

In this connection the observations of Perkins ('02) are of interest. He discovered that the hydriform larva of *Gonionemus* when kept in the laboratory lost its typical form and assumed an irregular amœboid shape. It moved about, apparently ingested food, repeatedly underwent a form of fission, and lived for over 2 months.

Obviously, therefore, viability and capacity to develop are by no means synonymous, and we have the theoretical possibility of the existence of persistent larval forms due to unfavorable conditions as well as to genetic variations.

Other larvæ were replaced from KCN *n*/100,000 to sea-water at 48 hours, from the blastula stage. After a further 48 hours, many had formed early plutei. These were mostly of a very wide-angled type (Fig. 9), thus showing what Child finds in similar circumstances, and has called "differential recovery."

Thus between the 48th and the 72nd hour in the solution the larvæ had lost their power of recovery in sea-water, though not of continued existence.

[1] The molecular concentration is simply given for convenience: the actual ionic concentration of CN would naturally vary with the hydrogen-ion concentration and other factors of the sea-water used.

B. $HgCl_2$. *n*/5,000, *n*/10,000, *n*/20,000, *n*/50,000 and *n*/100,000 solutions allowed segmentation to proceed to the 2-, 4-, or 8-cell stage in 24 hours (mostly to the 4-cell stage). Many segmenting eggs, however, were obviously damaged, and by 48 hours all were dead. The course of events was similar in a *n*/200,000 solution, except that by 24 hours segmentation had proceeded to the 4- to 32-cell stage (mostly to the 8-cell stage). Transference from *n*/100,000 and *n*/200,000 to sea-water at 24 hours had no effect, all being dead at 48 hours. The dead blastomeres were well-preserved, not cytolysed or disintegrated.

As the solutions used were obviously too strong, 24-hour gastrulæ from the control were placed in *n*/500,000 and *n*/1,000,000 solutions. These too were all dead 24 hours later (48 hours from fertilization); but they showed the interesting phenomenon of differential disintegration. All the tissues except the archenteron had entirely disintegrated, and lay as a sheet of cell-débris on the bottom of the vessel. The archenteron, on the other hand, was well preserved, and the outlines of its walls could be clearly seen (Fig. 10). The spicules were usually to be seen adherent to it. Sometimes it appeared solid. This is obviously an occurrence of the nature of those observed by Child ('15), and utilized by him in his axial gradient theory.

3. Dedifferentiation of Larvæ.

Unfortunately no further *Echinus* were to be had, and I was therefore unable to repeat the experiments with more suitable strengths of solution. Wishing, however, to see what the effect of poisons might be on more advanced stages, I transferred some of the plutei from the controls to various solutions of KCN and $HgCl_2$. The most interesting results were obtained in the mercuric-chloride solutions.

C. A preliminary experiment was made with 2-day plutei in $HgCl_2$ *n*/1,250,000. After 6 hours, many showed a retraction of the arms, leaving part of the skeletal spicules protruding. After 24 hours, many had died. The following types could be distinguished:

1. Partially dead, with disintegration of the tissue of the arms; the aboral ectoderm not disintegrated, or with a few cells migrated

out of the tissue and adherent to it externally (these cells appeared normal, in contradistinction to the pathological granular disintegration of the tissue of the arms). The form of the gut was well preserved, and no disintegration of any sort was visible in it (*cf.* Section 2, *B*).

In some cases it appeared that all tissues had disintegrated with the exception of the somewhat contracted gut, which was thus left by itself; but I was unable to be quite sure of this.

2. Slightly shrunken plutei with the skeleton protruding through the arms; these latter might be from 25 to 75 per cent. of their original length.

3. As (2) but the skeleton not protruding from the arms. The terminal portions of the spicules had apparently been resorbed by the arms as they contracted.

All the plutei had sunk to the bottom, and ciliary action was so much reduced that only very sluggish movement was taking place.

In addition to the types mentioned, others were seen which were of spheroidal form, dense, without arms, and with simple or no spicules. I at first thought that these might be examples of extreme dedifferentiation, but on examining the control culture, I found at the bottom a certain proportion of quite similar organisms. The culture was slightly overcrowded and these were doubtless susceptible individuals inhibited by the slightly adverse conditions. These types are mentioned to draw attention to the necessity for careful control in similar experiments. (This has already been emphasized by Shearer, De Morgan and Fuchs ('13), p. 274, etc.) They are also interesting as indicating that considerable inherited variations in vigor and resistance occur among the offspring of a single pair of *Echinus*.

In the later experiments now to be recorded, the plutei were picked out from the control culture with a fine pipette under the binocular microscope, transferred to the medium to be tested and there examined, to see if any of these minute armless forms had been transferred by mistake. In no experimental vessel was more than one of these forms discovered immediately after transfer; it can therefore be safely assumed that in this series the experi-

mental animals were for practical purposes all healthy plutei with well-developed arms.

D. Four-day plutei transferred to *n*/3,000,000 and *n*/4,000,000 $HgCl_2$. After 24 hours, all appeared normal, and about 90 per cent. were still swimming. After 48 hours, all had sunk to the bottom, ciliary action was somewhat reduced, there was a slight general shrinkage and loss of transparency as compared with the controls, and the arms had become slightly though definitely shorter, but without protrusion of the skeleton. The experiment was here discontinued, after another series had been started.

E. Five-day plutei (*a*) to $HgCl_2$ *n*/1,000,000, (*b*) to $HgCl_2$ *n*/2,000,000, (*c*) control in same amount of outside sea-water.

The controls remained healthy, with long arms, throughout the first 4 days of the experiment. The results may be tabulated as follows:

TABLE I.

	(*a*) $\frac{n}{1,000,000}$.	(*b*) $\frac{n}{2,000,000}$.
24 hrs.	All at bottom. Marked arm-resorption; no protrusion of spicules; arms mere knobs or ¼ to ½ normal length; aboral end usually swollen.	All at bottom. Moderate arm-resorption; no protrusion of spicules.
48 hrs.	Still further arm-resorption.	Slightly more resorption than at 24 hours.
72 hrs.	Arms absent or only knobs; a few with total dedifferentiation of oral end; many with aboral end no longer swollen, mesenchyme clumped, gut shrunken, spicules sometimes just protruding.	Slightly more resorption than at 48 hours.
96 hrs.	Spicules often protruding, most showing disintegration at oral end, and narrow form of body.	Condition as in (*a*) after 24 hours.
120 hrs.	Dead.	Condition as in (*a*) after 48 hours.
144 hrs.		Condition as in (*a*) after 96 hours.
168 hrs.		Dead.

(*a*) *n*/1,000,000. Some individuals after 24 hours in the medium are represented in Fig. 1.

The controls had arms about 25 per cent. longer than Fig. 1, *c*. It will be seen that in all cases the arms have diminished in length. They have also become more dense in appearance, being in the more advanced examples quite opaque. The diminution is rarely less than that seen in Fig. 1, *c*, and ranges from about 20 per cent. to about 80 per cent. The skeleton does not protrude at the tips of the arms, and the missing parts have apparently been resorbed. Another marked feature in the large majority of the specimens is the dilatation of the trunk and aboral regions (Fig. 1, *a–c*), which leads to a bulgy form, usually with a wide separation of the aboral ends of the spicules.

This may be explained as an effect of differential susceptibility, the more susceptible oral region contracting before the aboral, which then becomes distended with fluid. In some specimens (Fig. 1, *c*) the trunk ectoderm was seen to be definitely thinner than normal, implying distention. Other specimens (Fig. 1, *d* and *e*) did not show these phenomena at all markedly; in every case, such non-distended individuals exhibited extreme arm-resorption, and were also well below the average size. These were presumably individuals of general low susceptibility, in which all regions had suffered, the aboral ectoderm also being contracted, and the total volume of body-fluid being in some manner diminished. In point of fact, they were usually seen (Fig. 1, *d*) to have abnormally thick aboral and trunk ectoderm.

The number of pigment-granules appeared in general to have increased.

The appearances after 48 hours were very similar, with an increase in the amount of arm-resorption, and of aboral contraction.

After 72 hours, none had more than quite vestigial arms (Fig. 2, *a*), while the majority were almost or quite armless (Fig. 2, *c*). Very few now showed the separation of the aboral ends of the spicules, and not so many the swelling of the trunk region (Fig. 2, *c*). Many, on the contrary, were very narrow, especially at the aboral pole, and showed a condensation of the mesenchyme cells to form clumps (Fig. 2, *a*). In Fig. 2, *b*, the aboral region of the same specimen is shown under a higher power. The large clumps of mesenchyme cells are extremely like the clumps of

blood (mesenchyme) cells seen in dedifferentiated specimens of the Ascidian *Clavellina* (*cf.* Driesch, '06).

Up till 48 hours, the gut had remained apparently normal. In these specimens, however, though the stomach was of normal (Fig. 2, *a*) or even more than normal size (Fig. 2, *c*) the œsophagus and intestine showed some degree of contraction.

Extreme cases of dedifferentiation at 72 hours are shown in Figs. 2, *d* and *e*. In Fig. 2, *d*, the general turgescence or tone, and the transparent appearance of the aboral region is preserved, but the oral region has rounded off, and all traces of the characteristic form of the oral half of the animal, including the ciliated band, have disappeared. The stomach too is affected, all parts of the gut being now reduced, with thick epithelium.

Finally in Fig. 2, *e*, we have a specimen with complete dedifferentiation of the oral region. The aboral region, however, is also markedly affected, and the whole body is filled with a nearly opaque mass of cells, within which only faint traces of organs are visible. The skeleton is reduced to two simple clubbed rods. In this condition the animal much resembles a dedifferentiated *Clavellina*, and the resemblance would externally be almost complete if it were not for the presence of the spicules, which prevent the retraction of the aboral region and the assumption of the spheroidal form.

At 96 hours, most of the specimens showed signs of partial death. This manifested itself in the oral region by a disintegration of some of the tissue, and the protrusion of the skeleton. The gut was in all cases contracted. The aboral region was usually contracted, but with no trace of disintegration. (Fig. 3, *a* and *b*.) Most of them were transferred to sea-water. Those that remained in the solution were all dead after 120 hours, with aboral as well as oral disintegration, but very little or no disintegration of the gut. Slight movement due to ciliary action, and occasional contractions of the œsophagus were seen up to 96 hours.

One interesting occurrence was noted after 72 hours. Two plutei had grown together, the left anal arm of one having completely fused with the aboral region of the other, the skeletons of the two individuals overlapping in the common region (Fig.

4). The pair was isolated in sea-water, but was dead by 120 hours.

The progress of dedifferentiation in $HgCl_2$ *n*/2,000,000 was similar but considerably slower.

This is shown in Fig. 5, which represents an advanced specimen after 120 hours (by which time all those in *n*/1,000,000 were dead). An abnormal addition to the skeleton is seen. This may perhaps be due to the deposition in the aboral half of calcium carbonate resorbed in the arms, or to the fact of the spicule-secreting tissue being less affected than the rest, as Child ('16) suggests. The mouth was, I think, closed; the œsophagus was swollen. Most individuals after 120 hours were in the same stage of dedifferentiation as shown by the *n*/1,000,000 culture after 48 hours.

Partial death was not seen till 144 hours; complete death had occurred by 168 hours. As in the *n*/1,000,000 culture, the still-living portion when partial death had set in was very narrow, the spicules being nearly parallel. This narrowness is a result of all regions being affected simultaneously.

The controls for this experiment remained very healthy and off the bottom for 96 hours. The culture was rather crowded and not fed. After 120 hours, most were on the bottom, and were showing a certain amount of arm-resorption (though less than that shown in *n*/1,000,000 after 24 hours). One was seen with 3 arms reduced to minute knobs, but the fourth perfect. This was never seen in the $HgCl_2$ cultures, although there the arms of one side might be resorbed faster than those of the other.

The resorption in the controls might have been due either to starvation, or to the accumulation of toxic products, or both, but tests were not made to determine the point. Runnström's results ('17), show that either alone can cause dedifferentiation.

All were on the bottom after 144 hours. They remained in this position with almost total absence of arms, but healthy in other respects, for 5–7 days longer.

F. Nine-day plutei from the main control culture (fed on *Nitzchia*) were transferred to $HgCl_2$ *n*/1,000,000.

The appearance of the more advanced of these is shown in Fig. 6. All had developed the third pair of arms.

After 24 hours all had become somewhat reduced in size, with slightly denser appearance. The gut too was shrunken. The arms, especially the third pair, usually showed slight reduction in size.

After 48 hours, marked changes had occurred (Fig. 7). The third pair of arms had in all cases totally disappeared, and the others showed considerable but variable resorption. The previously noted swelling of the trunk and aboral region was present to a greater or less degree. The most interesting change was seen in the gut, which was always contracted, and contained a greater or less number of small round bodies. On examination with a higher power, these proved to be cells, practically spherical, and not cohering. They had presumably migrated out of the stomach epithelium into the lumen.

This is paralleled by the migration of the cells out of the aboral ectoderm in Expt. *C* above (pp. 212–213), and by the behavior of the tissues in organisms that dedifferentiate by resorption, as in *Perophora* (Huxley, '21 *b*) and Hydroids. (Loeb, '00. Huxley and de Beer, in press.)

After 72 hours, dedifferentiation had progressed much further (Figs. 8, *a* and *b*). The size is much less, the arms very small and extremely dense, the gut quite packed with cells, and much contracted. The contraction of the gut has expelled some of the cells at the anus and sometimes also at the mouth. The trunk and aboral regions are sometimes swollen, more usually contracted. A fair number of plutei had died.

At this stage the survivors were replaced in sea-water.

KCN. Experiments on 4-day plutei in KCN *n*/100,000 and *n*/200,000 gave on the whole similar results to those in $HgCl_2$ *n*/1,000,000. Disintegration of the trunk ectoderm was never observed, and is possibly a specific effect of Hg (*cf.* Child, '17). Arm-resorption was not quite so rapid.

4. Experiments on Recovery.

These are very incomplete, owing to lack of material. Through the courtesy of Mr. J. Gray, who independently observed dedifferentiation phenomena, in larvæ treated with citrates, I am enabled to state that in this case some degree of recovery at least is possible. It would here appear that forms which have resorbed

their arms (similar to my Fig. 1, *b*, or 1, *d*) are able to regenerate them, while those which have dedifferentiated to a completely spheroidal mass can probably redifferentiate to a pre-pluteus (helmet-shaped) stage at least. Mr. Gray, however, proposes to investigate the matter further, and I merely quote his preliminary results in order to show that dedifferentiation does not preclude recovery, at any rate to some degree. As is well known, dedifferentiation in *Clavellina* and in *Protozoa* does not preclude complete recovery (Driesch, '06; Lund, '17, etc.).

The observations I have made, however, on forms dedifferentiated by KCN and $HgCl_2$ (in which case dedifferentiation appears to be slower than when caused by citrates) have not yet shown recovery, though many specimens lived for weeks in sea-water, and ingested food. From the present results, it would seem as if the poison had robbed the organism of its capacity for development, but not of its capacity for maintaining life. I must emphasize that owing to lack of more ripe *Echini,* I was unable to do more than carry out preliminary observations, and that systematic experiments on the subject are in view.

That being so, I will record my results in the briefest possible manner. Most moderately-dedifferentiated forms (*i.e.*, with ciliated band, but arms absent or vestigial) transferred to sea-water from KCN remained alive and in approximately the same condition for over 4 weeks. They kept on the bottom, and retained a slight motility. In one culture the beginnings of recovery were noted in the shape of increased motility and of a few larvæ beginning to swim freely once more, but after a few days they again reverted to their former condition. In all cultures a slight *progressive dedifferentiation* was noted after 1 to 2 weeks, and towards the end of the 4 weeks, a larger proportion of spheroidal forms was found.

On adding *Nitzchia* to two cultures, it was found that diatoms were ingested by many of the dedifferentiated larvæ. These cultures lived better than those without *Nitzchia.*

A number of these larvæ were also placed, after 2 weeks, in a jar with *Nitzchia* and a stirring apparatus; but 3 weeks later none could be discovered.

The history of those transferred to sea-water from $HgCl_2$ is

similar in essentials. The larvæ lived up to 3½ weeks. Some ingested *Nitzchia* when this was provided. Progressive dedifferentiation was, I think, more pronounced in these $HgCl_2$-treated larvæ.

Figures are given of some of the types seen during this progressive dedifferentiation. Fig. 11 shows a larva which has completely lost its arms and also its ciliated band, together with the antero-lateral and transverse skeleton on one side. The remainder of the skeleton permits it to retain some of its characteristic form. The gut and its epithelium are contracted, and its spatial relations altered. The body-cavity is clear, with a few clumps, some of pale cells, others of red pigment-cells. The general appearance is much clearer and less full of cell clumps than in larvæ in advanced stages of dedifferentiation still in the toxic solution.

Fig. 12 shows a further stage of loss of form. Here the outline is simply spheroidal. The broad œsophagus, contracted stomach and thin intestine lie approximately in a straight line. The body-cavity is very clear, with the exception of a few large clumps of cells. The aboral clubs have been broken off from the rest of the skeleton. This breaking of the skeleton, it should be noted, was frequently seen in forms where the aboral and trunk regions were much dilated. The change of form of the aboral region exerts a pressure inwards on the rods, at right angles to their axis, and snaps the ends off; at first they lie in the position in which they have been broken, thus making it easy to see how breakage occurred. Once this happens, the oral ends of the rods will no longer be pressed against the body wall, and the whole skeleton thus ceases to function as a support. This is clearly seen in Fig. 12, *a* and *b*.

The general appearance of the tissues in this larva, and indeed in most of those in the recovery experiments, is perfectly healthy. Even after the skeleton has disappeared and the spheroidal form has been assumed, traces of the "lip" or pre-oral lobe may often be seen near the mouth. This also, however, has disappeared in the larvæ shown in Fig. 12.

A very advanced stage of dedifferentiation is seen in Fig. 13 *c*. The larva had shrunk considerably in size. This is apparently due to the contraction of the ectoderm, which was cuboidal in-

stead of flattened. No trace of skeleton was present. (The animal was examined both *in vivo* and as a stained preparation.) Large, brownish aggregations were seen in the interior, together with an apparently closed and solid pale vesicle, perhaps the stomach. Curious irregularities of the ectoderm were observed at one pole. These were also seen in several other specimens (*cf.* Fig. 12 *b*).

The general resemblance of this specimen to much-dedifferentiated individuals of the Ascidian *Clavellina* is striking (Driesch, '06; Schultz, '07; and my own unpublished observations). There are numerous points of difference, as one would expect in such widely different organisms, but the following essential similarities exist: (1) the assumption of the spheroidal form; (2) the aggregation of free cells to form dark masses; (3) the regression of epithelial cells to the cuboidal condition; (4) the conversion of internal structures into closed vesicles; (5) the congested condition of the body spaces, consequent upon contraction.

5. On the General Effect of Mercury in Dilute Solutions.

In order to get some more accurate idea of the processes occurring in a weak solution of a mercury salt, some experiments were carried out on the gill of *Mytilus*. I have to thank Mr. J. Gray, of King's College, Cambridge, for some suggestions.

A preliminary test with various strengths of $HgCl_2$ solution showed that in very weak solutions marked disintegration of the tissue took place before ciliary action was stopped.

The point to be tested was whether the effect of Hg^+ ions was proportional to the *strength of solution* used, or was a progressive effect, proportional to the *total amount* of mercury in the solution.

(*A*) Five finger bowls were prepared, and 4 pieces of gill placed in each. One contained 50 c.c. of sea-water as control; the others 50 c.c. of $n/375{,}000$ $HgCl_2$. The solution in Nos. 1 and 2 was not changed. In No. 3, it was changed after 2, 4, 6, 8 hours and again after 24 hours. In No. 4, it was changed every ½ hour for 8 hours, and again after 24 hours.

After 3 hours, there was slightly more disintegration in No. 4 than elsewhere. After 6 hours, all the pieces in No. 4 had their

terminal rounded portion markedly disintegrated, and the current produced by the lateral cilia had ceased.

In Nos. 1 and 2, one piece in each was similar, but the others had scarcely begun to disintegrate. No. 3 was intermediate. In Nos. 1, 2, and 3 the current produced by the laterals was still evident.

After 24 hours, No. 4 was very markedly disintegrated, and no ciliary movement was visible. In Nos. 1 and 2, 4 of the 8 pieces showed ciliary movement, and the disintegration was not so marked as in No. 4. No. 3 was intermediate. The conclusion was apparently to be drawn that it is the total amount and not the concentration of the Hg^+ ions present that is operative; but the concentration used was apparently too high. The experiment was therefore repeated in a modified form: (*B*) Five finger bowls were prepared with 5 pieces of gill in each. In them were placed respectively 5, 20, 50, 100 and 300 c.c. of a $n/1{,}500{,}000$ $HgCl_2$ solution.

The following table gives the results observed (+ = strong ciliary action; ⊕ = moderate; ⊕ ⊕ = faint; ⊕ ○ = very faint; ○ = no ciliary action. ((D)) = slight disintegration; (D) = medium; D = marked disintegration.

TABLE II.

No.	c.c.	Time in Hours. 3	21	27	45	70	
1.....	5	+	+	+	⊕	⊕	All proximal cut ends healed.
2.....	20	+	+	+	⊕	⊕ ((D))	
3.....	50	+	⊕ ((D))	⊕ (D)	⊕⊕ (D)	.....[1]	All proximal ends unhealed; dead after 45–70 hrs.
4.....	100	+	⊕ ((D))	⊕⊕ D	⊕⊕ D	.....[1]	
5.....	300	+	⊕ ((D))	⊕⊕ D	⊕⊕ D	⊕○ D	

It will be seen that the effect of the $HgCl_2$ was a function of the total amount used, all the solutions being of the same strength. The death of the proximal portions of the filaments was very striking in the large bulks of solution.

[1] Numbers 3 and 4 were not observed at 70 hours, owing to an oversight.

There was, however, very little difference between the effect of Nos. 4 and 5. That is to say, the maximum effect possible with a solution as weak as $n/1{,}500{,}000$ is, with the amount of tissue used in the experiment, attained with a total bulk of about 100 c.c.

In any experiments concerning the effects of salts of the heavy metals upon living cells, it will therefore be necessary in every case to consider not only the strength but also the amount of the solution, and further the amount of living tissue on which experiments are being carried out. Failing this, the results obtained will not be comparable. However, since in the experiments on plutei here recorded, the bulk of $HgCl_2$ solution was large in comparison to the bulk of the plutei, and since the results are only qualitative, they are not invalidated by the conclusions just reached.

6. Discussion.

A. *Maintenance of Form.*

Recent work is coming to show more and more clearly how organic form is the product of an equilibrium between constitution (internal forces) and environment. This applies equally well to whole organisms or to their parts. In order that the typical form may be maintained, a particular complex of environmental stimuli is necessary.

In the case of Ascidians such as *Clavellina* (Driesch, '06; Schultz, '07) and *Perophora* (Huxley, '21 *b*) whole zoöids below a certain size when exposed to unfavorable agencies, such as accumulated waste products or dilute solutions of KCN, are unable to maintain their form, and undergo what is known as dedifferentiation.

The same is true of Hydroid polyps (Loeb, '00). In Hydroid polyps we (Huxley & De Beer, in the press) have succeeded in finding a quantitative relation between the rapidity of the form-changes and the concentration of the toxic solutions employed; this is also seen in the present study (Table I.).

The encystment of *Protozoa* in times of drought or cold is an example of the same phenomenon.

The remarkable dedifferentiation of the hydriform larva of

Gonionemus observed by Perkins ('02) should again be mentioned here.

As regards parts of organisms, attention may be called to the phenomena seen in sponges (Minchin, '00, p. 29). When an Ascon type of sponge contracts, the internal environment is altered, and the collar cells are unable to maintain their typical form, losing their collar and flagellum and becoming spheroidal. See also Huxley ('21 *a*, p. 313).

The familiar resorption of many grafted tissues may also be mentioned. Muscle fibers, like *Clavellina*, also dedifferentiate preparatory to regeneration when cut across. (Towle ('01).)

Even the form of mental organization is subject to the same limitation. In certain "shell-shock" and other cases, strain and unfavorable environment render the higher part of the mental organization unable to maintain itself, resulting in what is known as *regression*. See Nichol ('20).

We may thus say that maintenance of normal form is possible only in certain environments. Certain stimuli result in what may be called hyper-typical form: *e.g.*, in regenerating Planarians, high temperature produces forms with exaggerated heads (Lillie and Knowlton, '97; Child, '15, p. 138). On the other hand, many unfavorable stimuli do not permit the establishment of the type at all: they result in infra-typical form—*e.g.*, cold in regenerating Planarians; below a certain temperature, no head is formed (*auctt: cit.*).

B. *Dedifferentiation and Metamorphosis.*

The resemblance of the phenomena here described to those occurring at the metamorphosis of the pluteus is very striking. According to MacBride ('02) the course of the process is as follows: The larva sinks to the bottom, presumably as a direct result of the weight of the growing *Echinus* rudiment. The arms are next resorbed, those on the same side as the *Echinus* rudiment first. The larval œsophagus contracts. The ectoderm of the ciliated epaulettes is "invaginated" and devoured by amœbocytes (the description, however, does not negative the possibility that the cells of the epaulettes may migrate out of the tissues, rather than amœbocytes migrate in). During arm-resorption, the ectoderm

of the arms shrinks, leaving the spines exposed "exactly as in unhealthy larvæ at all stages of development." The oral lobe and the outer part of the œsophagus disappear. The inner part of the œsophagus persists for a time as a completely closed tube. Very remarkable changes occur in the stomach. In the late larva it is highly turgid, with cells intermediate between cubical and flattened. It now loses its turgidity; the walls become very thick, and eventually folded, the lumen often almost disappearing. The cells are stated to multiply with great rapidity, but definite proof of this is not given, and quite possibly the appearance of increased number is due to the contraction of the wall; this point deserves re-investigation. The cells round themselves off, and many migrate into the surrounding jelly. The new stomach is reconstituted from the residue.

While this has been occurring, all resemblance to an Echinopluteus form has disappeared, and the animal becomes almost hemispherical.

The essential points to be noticed are as follows:

(1) The resorption of the arms. This appears to be identical with what we have seen in larvæ placed in toxic solutions.
(2) The dedifferentiation of the specialized larval organs, the epaulettes. These are not present in the earlier larvæ used by me.
(3) The loss of the general form of the larval part of the organism, and its approximation to the segment of a spheroid.
(4) The contraction of the œsophageal tissue.
(5) The closure of the mouth and formation of a closed vesicle from the remains of the œsophagus.
(6) The contraction (loss of turgidity) of the stomach.
(7) The migration of some of the cells of the stomach out of the tissues. This was paralleled in my experiments, though there the cells migrated inwards to the lumen, instead of outwards to the body-cavity.

As far as the destruction of larval organs goes, we can assert that the dedifferentiation caused by toxic solutions and the reduction at metamorphosis are closely and essentially similar. The difference between the end-results is presumably due to the fact

that at metamorphosis there is present a new organic system, the developing *Echinus* rudiment, which was absent in the subjects of the dedifferentiation experiments. We may suppose that the metamorphic changes in Echinoids are normally initiated somewhat as follows: the inherited constitution of the animal leads to the production of the *Echinus* rudiment. The weight of this leads directly to the sinking of the larva to the bottom. The subsequent changes would then be due to two causes: (1) The conditions at the bottom are directly unfavorable to the larval organs, which therefore are unable to maintain themselves, and so start dedifferentiating. (2) The developing adult organs are not inhibited by the benthic environment, and their continued growth and consequent demands for nutrition accelerate the dissolution of the larval system.

The stomach is an organ which is remodelled. It is unable to maintain itself in its typical larval form, and regular dedifferentiation-changes start in it. But the activities of the adult organs provide a new internal environment, and the remains of its tissues, entering into equilibrium with this, form the rudiment of the adult stomach.

It should follow from these considerations that precocious metamorphosis should be induced by placing larvæ with a developing *Echinus* rudiment in dilute toxic solutions. This conclusion it is intended to test by experiment. Meanwhile Professor MacBride informs me in conversation that those of his cultures in which conditions are not optimum, do as a matter of fact exhibit precocious metamorphosis. In such conditions, the larvæ sink to the bottom while the *Echinus* rudiment is still in a stage much less advanced than that which it possesses at metamorphosis in the best cultures. In spite of this, metamorphosis takes place, but leads to the production of small, weakly, under-developed Echini. I am grateful to Professor MacBride for informing me of this confirmation of my theoretical considerations.

Loeb ('00) made the suggestion that the retrogressive changes of histolysis in metamorphosis were comparable to dedifferentiation as seen in Hydroids, but I am not aware that the similarity between dedifferentiational and metamorphic changes in one and the same species has yet been pointed out, as here in *Echinus*.

If these considerations prove to hold good, it will follow that phagocytosis is a secondary phenomenon in the metamorphosis of Echinoids, and probably of other groups. It will, however, obviously be our next task to test by experiment the hypothesis that the dedifferentiation of larval tissues is the essential factor initiating Echinoid metamorphosis.

C. *Axial Gradients and Surface-effects.*

Child's theory of axial gradients and the differential susceptibility along them has been set out at length in his books ('15, etc.) and papers, and it is unnecessary to enter into it here.

I would like to point out, however, some facts which may lead to a modification of some minor parts of the theory.

Child measures and delimits his "metabolic gradients" by means of the differential susceptibility of different organs to toxic agencies. In certain cases this gives concordant and uniform results. In other cases the susceptibility of an organ is found to vary during development in a way which is not to be explained without demanding a very considerable elasticity from the theory.

The general bases of the theory appear to be founded on solid enough foundations, the main difficulty being that they are perhaps too general, the term "metabolic rate," for instance, being only capable of application in an unanalysed sense, as an expression of general total activity. But numerous exceptions, such as the unpredictable variation in susceptibility of organs above alluded to, can I think be explained by reference to another and simpler notion. That is, that cells are more susceptible and more prone to dedifferentiation in proportion to the amount of surface which they are exposing. We can put this in another way, and say that a cell maintains its form with greater difficulty when its surface is large than when it is small.

This statement is based on numerous facts, including the following observed by myself, as well as being deducible from theoretical considerations.

(1) In the Ascidian *Clavellina,* the parts first showing dedifferentiation, and finally most dedifferentiated, are those where the normal cells expose a great deal of surface (pharynx and atrium).

(2) The same is true of the related form *Perophora,* in spite of its different mode of dedifferentiation.

(3) In *Perophora,* the stolon-ectoderm is usually flat. When treated with toxic agents of a certain strength, it becomes cuboidal.

(4) In sponges, the collar-cells exposed to very mildly toxic agents retract their collars. Further toxicity causes the assumption of the spheroidal form by the cell. (Huxley, '21*a*.)

(5) In dissociation experiments, the shock of mechanical separation causes the assumption of the spheroidal form, and the loss of any differentiated structures such as collars, flagella, or pseudopodia, in all types of cell in sponges. (This is true also in other sponges (H. V. Wilson, '07) and in Cœlenterates (H. V. Wilson, '11; De Morgan and Drew, '14).)

(6) In the plutei here described, the gut is at first extremely resistant to toxic agents. Later, however, it becomes very susceptible to them. This alteration in susceptibility is accompanied by an alteration in appearance, the gut passing from a thick-walled organ to an extremely turgid, thin-walled one. This turgidity has also been noted by MacBride ('02); the cells in this latter condition are very much flattened, with a relatively enormous surface. One of the first effects of $HgCl_2$ solutions in later larvæ is to cause a shrinkage of the gut and a separation and rounding-up of some of its cells.

It is of course presumable that high energy-consumption is necessary to maintain a cell in a flattened condition, or in any other involving a large surface area—as would indeed be expected from the laws of surface-tension. But to say that a high "metabolic rate" thus produced is to be reckoned in the same category as the high metabolic rate of a "dominant" region as defined by Child, is to reduce the value of the whole very important conception of physiological dominance.

Further, in cells grown in vitro, Holmes ('14) notes as a general rule that any unfavorable condition leads to the abandonment of an extended for a spheroidal shape. Numerous other instances could be cited, but the phenomenon is so widespread as to be familiar to all.

We may therefore say that, apart altogether from the question

of axial gradients, the susceptibility of cells to toxic agents, and their readiness to dedifferentiate, are in part functions of their surface area.

D. *Previous References to Dedifferentiation in Echinoid Larvæ.*

Vernon ('94) found that various environmental factors had a marked effect on the arm-length of plutei (the experiments were continued up to the 8-day stage). The effect, whether caused by actual resorption of tissue present, or failure to grow beyond a certain length, means that equilibrium with these slightly unfavorable conditions could only be maintained by arms of a shorter length than normal.

Robertson ('13) found that *Strongylocentrotus* blastulæ transferred to a medium containing 0.15 per cent. lecithin for 24 hours and then returned to sea-water, not only were very much retarded in development, but after becoming gastrulæ nearly lost their gut again before dying (figures and details are not given). He ascribes this result to a specific action of lecithin on growth-processes. From what we know of Echinoderm development, the result, in the absence of further data, might at least equally well be non-specific and due to dedifferentiation.

In addition, the literature abounds with notices that "unhealthy" larvæ may show shortened arms, sometimes with a terminal protrusion of spicules. (And see postscript, p. 230.)

E. *Recovery.*

The meager results on recovery merit one or two words. Future experiment must decide between one or two possibilities. Either the Hg^{+} ion damages only a fraction of the cells' protoplasm, which, if it does not exceed a certain critical value, may be repaired, and normal differentiation resumed; or repair is not possible, and the animal cannot differentiate further. The theoretical bearings of this second possibility have been discussed by Child ('16) and I do not intend to go into them at present. What is interesting in either eventuality is the extremely long period for which the dedifferentiated organisms can remain not merely

alive, but, it should be emphasized, with apparently quite healthy tissues.

The fact that such larvæ ingested diatoms in a normal fashion is noteworthy. The possibility is thus raised of producing, by environmental changes, permanent larval forms. (*Cf.* Perkins, '02.) Such permanent larval forms of small size, but hereditarily determined, are of course known in many species, especially in the male sex.

The action of mercury is apparently to precipitate and put out of action a definite quantity of the living molecules in the cell.

Postscript.

Owing to delay in the arrival of German periodicals after the war, I did not see the important paper by Runnström ('17) until I had not only completed the work here recorded, but also written the paper. Runnström has demonstrated that young Echinoid plutei treated with $ZnSo_4$ in very weak solutions undergo dedifferentiation-changes very similar to those observed by me. He also finds that dedifferentiation occurs spontaneously in a certain proportion of larvæ in every culture, especially from over-ripe eggs. The specimens noted by me on p. 213 above are doubtless of this category. Finally, he describes very similar dedifferentiation as the result of starvation, from which recovery is possible. Recovery is also possible in the $ZnSo_4$ larvæ when replaced in sea-water.

I will not summarize his results further, except to say that they show conclusively that dedifferentiation may be produced by many agencies, and may be reversible.

There are one or two points of general theoretical interest which may be noted. While remarking on the resemblance of the changes seen at metamorphosis to those produced by starvation, Runnström says that an *essential* difference between the two is the rapidity of the metamorphic changes. On the other hand, reduction by means of toxic agencies is more rapid, and it should be remembered that at metamorphosis the existence of rapidly growing imaginal organs will drain the rest of the organism very quickly. The rapidity of dedifferentiation in the zoöid of *Perophora* is very great—*once it has started.* The observations of Professor MacBride (p. 226) also corroborate strongly the idea

that toxic agencies are important in the initiation of metamorphosis. Cytolysing agencies are not necessary to explain resorptive dedifferentiation such as occurs in Hydroids and *Perophora;* they should therefore not be postulated, as is done tentatively by Runnström, to explain metamorphic changes in Echinoderms until definite proof can be adduced of their existence.

A further corroboration of the idea of metamorphosis as an upset of balance is to be seen in Runnström's own remarkable observations upon the formation of pedicellariæ. In much-reduced larvæ, more or less well-differentiated rudiments of pedicellariæ often appear *ab initio*. Once pedicellariæ have been formed, however, they are among the first organs to be dedifferentiated. This may be interpreted by supposing that the potency of producing pedicellariæ is present in larvæ, but normally inhibited until a certain stage by the demands of the existing organs. When these are reduced, however, the check is removed and the pedicellaria develops. Once developed, on the other hand, its cells are not actively growing, and are easily affected by unfavorable agencies. This is very similar, it would seem, to the behavior of larval and adult organs at metamorphosis.

With Runnström's general discussion I find myself in full agreement in all essentials, except that I do not consider that he has laid sufficient stress upon the direct inhibiting effect of poisons upon growing tissues, an effect which leads eventually to dedifferentiation by a different route from that consequent upon hunger.

APRIL 6, 1921.

SUMMARY.

1. The retardation of early *Echinus* development caused by toxic agents (KCN and $HgCl_2$) is noted.

2. When recovery took place on transference to sea-water after 48 hours in the toxic solutions (*i.e.,* in the blastula stage), plutei were formed. These were mostly very wide-angled; this was due to the greater power of recovery of the oral region.

3. On transference to sea-water after 72 hours' treatment with the poisons (*i.e.,* in the gastrula stage), development only took place as far as the pre-pluteus stage. Considerable power of re-

covery is thus lost by prolonging the treatment with poisons from 48 to 72 hours.

4. Gastrulæ killed by weak toxic solutions show *differential death,* the enteron being more resistant than the rest of the tissues.

5. In very weak solutions, progressive *dedifferentiation of plutei* occurs. The arms are first resorbed, then the ciliated band and oral lobe disappear, then the gut contracts. In early stages, the trunk and aboral end are dilated, in later stages they are contracted and the whole body is filled with cells. The process is similar in all essentials to that seen in the dedifferentiation of the Ascidian *Clavellina.*

6. It could not be decided whether full *recovery* is possible for plutei thus treated. Armless forms replaced in sea-water remained alive and motile for 3½ to 4 weeks, and ingested diatoms. Some of them showed *further dedifferentiation* in the sea-water, finally reaching a spheroidal state, with spicules extremely reduced or absent, and gut reduced to a closed vesicle or vesicles.

7. The process of dedifferentiation appears to be essentially identical with that initiating *metamorphosis* and resulting in the destruction of the larval organs in Echinoids. This is probably true also for metamorphosis in other groups.

8. Echinoid metamorphosis, and possibly other types of metamorphosis also, would therefore appear to be *initiated* by dedifferentiation-changes in the larval organs, not by autolysis or phagocytosis, which are secondary phenomena.

9. Ceteris paribus, tissues with *large cell-surface* are more susceptible to unfavorable influences and more prone to dedifferentiation than are those with a small area of surface per cell.

10. The effect of mercury salts in dilute solutions is shown to depend upon the *total amount* present as well as upon the *concentration.*

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LIST OF FIGURES.

All figures were drawn from life with the aid of an Abbé camera lucida, at table level. All are drawn with the combination of a Reichert 1/3″ lens and a No. 4 ocular, with the exception of Fig. 2, *b* (1/6″ lens + 4 ocular), and Fig. 10 (⅙″ lens + 2 ocular, reduced to half-size). Figs. 6 and 10 have been reduced to one-half diameter, all others to two-thirds.

Plate I.

Figs. 1–4. 5-day plutei in $HgCl_2$, $n/1{,}000{,}000$.

Fig. 1. After 24 hours.

Fig. 1*a*. The most frequent type, with arms considerably resorbed, aboral end dilated. Anal view.

Fig. 1*b*. Similar, but arms more resorbed. The red pigment grains are here represented. Anal view.

Fig. 1*c*. Similar, but not quite so much arm-resorption; marked dilatation of the trunk-region. The epithelium of the trunk and aboral regions is exceptionally thin. Lateral view.

Fig. 1*d*. More complete dedifferentiation. The trunk and aboral regions are contracted instead of dilated; consequently the aboral ends of the spicules are not separated, and the trunk epithelium is thicker than normal. Anal view.

Fig. 1*e*. Similar, but with more pronounced arm-resorption. Anterior view.

Fig 2. After 72 hours.

Fig. 2*a*. Specimen with arms vestigial and dense, thin form, and clumping of mesenchyme. The stomach is normal, the intestine and œsophagus shrunken. Lateral view.

Fig. 2*b*. Aboral end of the same, under higher magnification. The light masses are composed of pale yellow cells with few granules. The dark bodies are red pigment. Quite aborally is a mass of slightly different clear cells.

Fig. 2*c*. The commonest type. The anal arms are totally absent, the oral arms and lobe almost entirely so. The skeleton of the oral arms is just protruding. The trunk and stomach are swollen, but the intestine contracted. Oblique anal view.

Fig. 2*d*. No trace of arms or of the form-differentiation of the oral end remains. On one side, the skeleton of the oral and anal arms has disappeared. The gut is shrunken, and its parts lie nearly in a straight line. Oblique anal view.

Fig. 2*e*. Complete dedifferentiation of the form of both oral and trunk regions. Body opaque and dense, filled with clumped mesenchyme. Skeleton reduced to two straight rods. Lateral view.

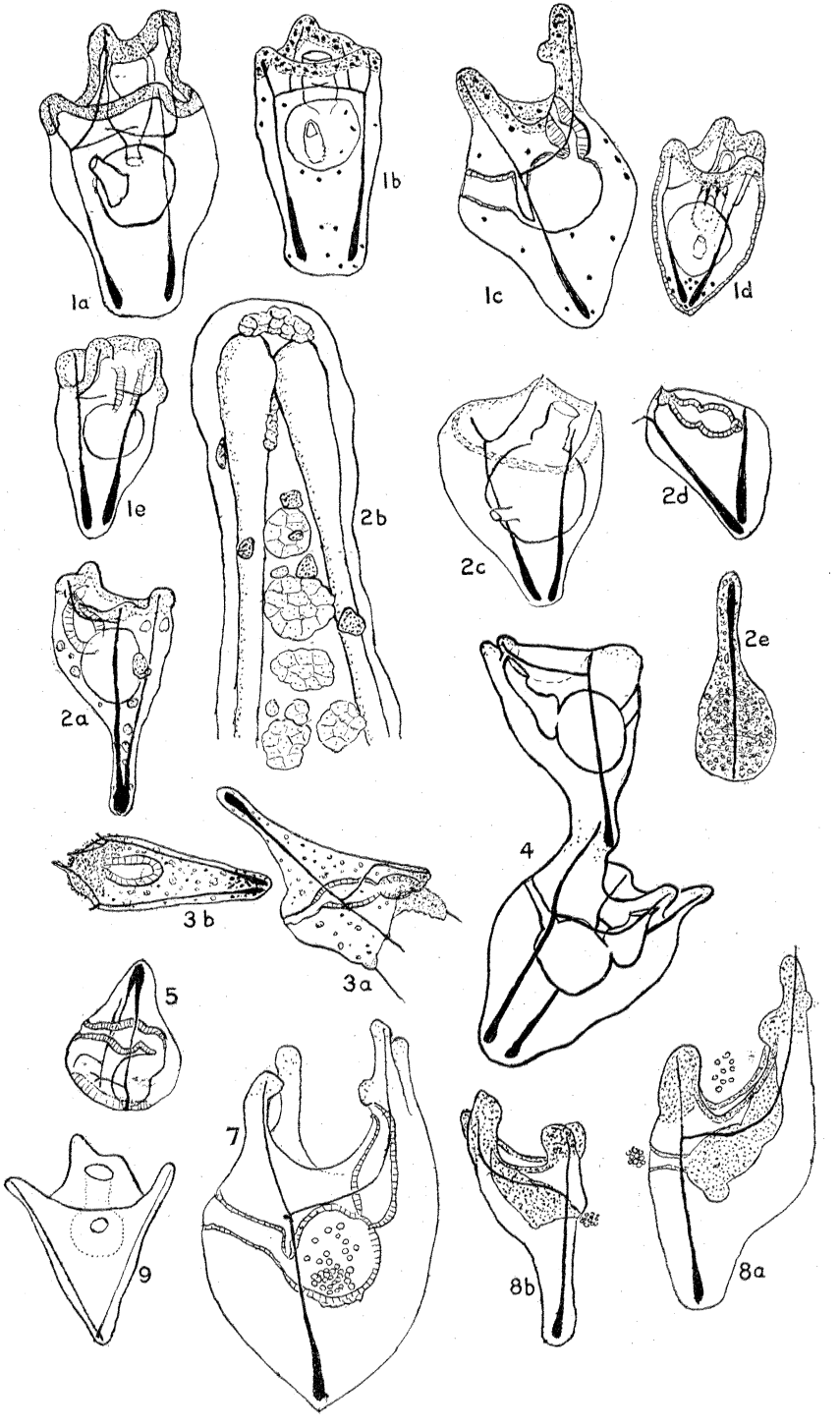
Fig. 3. After 96 hours.

Fig. 3*a*. Protrusion of skeleton, disintegration of oral arms only. Trunk region still somewhat expanded. Lateral view.

Fig. 3*b*. Specimen dedifferentiated in all regions, with incipient death-changes. A mass of red pigment aborally. Disintegration of whole oral region beginning. Anterior view.

Fig. 4. A pair of plutei found grown together after 72 hours. Lateral view.

Fig. 5. A 4-day pluteus in $HgCl_2$ $n/2{,}000{,}000$, after 120 hours. Note the remains of the ciliated band, the reduced stomach but dilated œsophagus, and the abnormal skeleton. Lateral view.



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FIGS. 6–8, AND 14. 9-day plutei in $HgCl_2$ $n/1{,}000{,}000$.

FIG. 7. After 48 hours, in $n/1{,}000{,}000$ $HgCl_2$. The third pair of arms has disappeared, the other arms markedly reduced; note the clubbing of one of the anal arms. The stomach contatins numerous cells migrated out of its walls. Aboral and trunk regions are swollen. Lateral view.

FIG. 8. After 72 hours in $n/1{,}000{,}000$ $HgCl_2$.

FIG. 8*a*. The arms are markedly dense, and very small. The skeleton of the oral arms protrude slightly. The remains of the oral lobe are visible. Stomach and œsophagus are quite dense. Cells have been extruded from both anus and mouth. The trunk is slightly swollen. Lateral view.

FIG. 8*b*. More advanced stage. The oral lobe has disappeared, the arms are dense and shorter, the trunk and aboral regions are affected. Cells have been extruded at the anus. Lateral view.

FIG. 9. Wide-angled pluteus. 2 days from fertilization in KCN $n/100{,}000$. 2 days in sea-water. Only the anal skeleton is drawn. Anal view.

PLATE II.

FIG. 6. Outline of 9-day control pluteus when transferred to the solution *n*/1,000,000 $HgCl_2$. The skeleton is omitted. Anterior view.

FIG. 10. Differential disintegration of gastrula in *n*/1,000,000 $HgCl_2$. The nature of the spherical non-disintegrated mass was not ascertained.

FIGS. 11–15. Stages of progressive dedifferentiation of plutei after replacement in sea-water from toxic solutions.

FIG. 11. Form without trace of ciliated band. Gut epithelium cuboidal (occasionally contracting). Trunk ectoderm thin. 2 days in KCN *n*/100,000, 17 days in sea-water. *m* = mouth.

FIG. 12. Completely spheroidal forms.

FIG. 12*a*. With aboral ends of spicules broken. Gut straight, with cuboidal epithelium. No trace of ciliated band, oral lobe, or typical form. Some branched mesenchyme cells. *m* = mouth. 2 days in $HgCl_2$ *n*/1,000,000, 10 days in sea-water.

FIG. 12*b*. Another specimen from the same vessel, 3 days later. Faintly motile. Gut reduced to a closed vesicle with cells aggregated round it. A curious projection with sharp lobes in oral region.

FIG. 13. Extreme reduction.

FIG. 13*a*. Non-motile, with remains of aboral ends of spicules, a dense mass of cells surrounding a gut-vesicle, and very thin ectoderm. 1 day in KCN *n*/25,000, 13 days in sea-water.

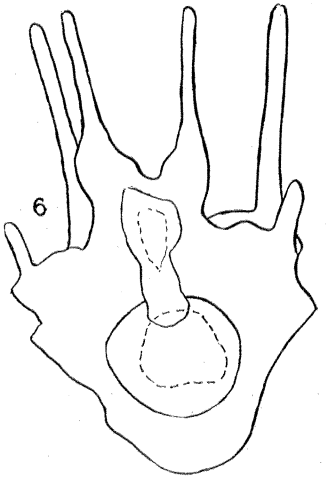
FIG. 13*b*. Still further reduction. Remains of only one spicule. Dense interior, with no visible trace of internal organs. 4 days in KCN *n*/25,000, 10 days in sea-water (with *Nitzchia*).

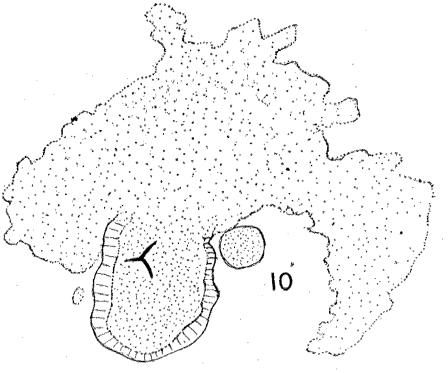
FIG. 13*c*. Faintly motile. No trace of spicules. Oral projection as in 12*b*. Several brown aggregations of cells, and a vesicle presumably derived from the gut. 2 days in $HgCl_2$ *n*/1,000,000, 30 days in sea-water (with *Nitzchia*).

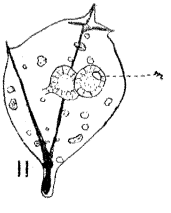
FIG. 14. A larva not quite so much dedifferentiated as 12*a*.

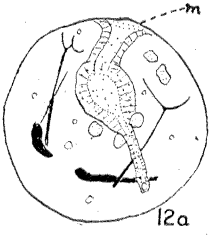
FIG. 15. A much-reduced larva, showing protrusion of the left spicule at both ends. Where it protrudes aborally, a ring of thickened ectoderm surrounds it. The other spicule is absent; presumably it has simply fallen out. The œsophagus has partially disintegrated. 3 days in $HgCl_2$, *n*/1,000,000, 2 days in sea-water.

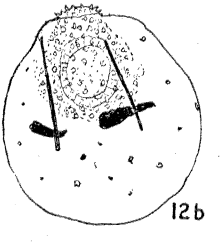
FIG. 16. A larva treated like that shown in Fig. 7.

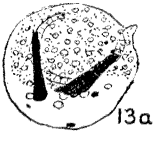


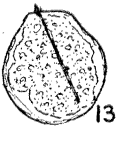


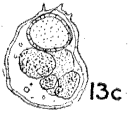


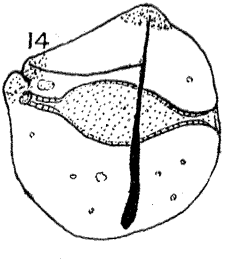


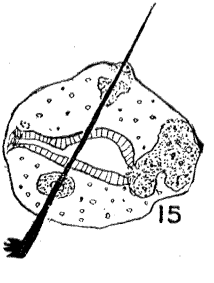


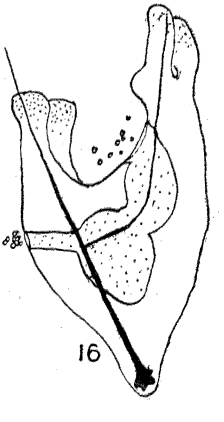












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